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## Foliar application of molybdenum reduces yield loss and pre-harvest sprouting in Japonica rice seeds subjected to simulated flooding during seed development and maturation

Sujitra Tejakhod<sup>1,2</sup>, John P. Hammond<sup>1</sup> and Richard H. Ellis<sup>1\*</sup>

<sup>1</sup> School of Agriculture, Policy and Development, University of Reading, Earley Gate, Whiteknights, PO Box 237, Reading, RG6 6AR, UK (E-mail: [r.h.ellis@reading.ac.uk](mailto:r.h.ellis@reading.ac.uk))

<sup>2</sup> Present address: Division of Rice Seed, Rice Department, Ministry of Agriculture and Cooperatives, 50 Phaholyothin Road, Ladyao, Chatuchak, Bangkok, 10900 Thailand

\*Author for correspondence

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### Abstract

Flooding damages rice crops and its incidence is increasing. Foliar spray applications of molybdenum (100, 600 or 3000 mg Mo L<sup>-1</sup>), abscisic acid (ABA, 50 µM), or deionised water (control) were made to pot-grown plants of the Japonica rice cv. Gleva at flag leaf appearance to determine their effects on seed yield and pre-harvest sprouting after flooding. Plants were submerged, to simulate flooding, for four days from 20 or 30 days after anthesis (DAA). Seed yield per plant, seed weight and pre-harvest sprouting were determined after harvest at 37 DAA. Seed yield and weight were substantially reduced and pre-harvest sprouting promoted by submergence, with greater sprouting after the later treatment. Prior treatment with molybdenum or ABA reduced pre-harvest sprouting ( $P < 0.05$ ), and possibly reduced damage to seed yield ( $0.10 > P > 0.05$ ), compared with deionised water. The molybdenum concentration in both sprouted and non-sprouted seeds harvested at 37 DAA responded positively to molybdenum application rate. Molybdenum application rate had no consistent effect on seed ABA concentration. Foliar application of 100 mg Mo L<sup>-1</sup> at 200 L ha<sup>-1</sup> provided the most promising mitigation of flood damage, but benefits were minor in the context of seed damage from submergence.

**Keywords:** abscisic acid, flooding, molybdenum, pre-harvest sprouting, rice, seed weight, seed yield

### Introduction

Pre-harvest sprouting, that is in-spike or on-panicle germination of seeds due to wet conditions before harvest, is influenced by the genotype and environment (rainfall/flooding and warm temperature) of the seed crop (Bewley and Black, 1994; Taiz and Zeiger, 2006; Sugimoto *et al.*, 2010; Bewley *et al.*, 2013). In rice (*Oryza sativa* L.), Wan *et al.* (2006) reported substantial yield losses due to sprouted grain with prolonged rain close to harvest. Some modern rice varieties have been particularly vulnerable; 20% of the

rice-growing area in Sichuan and the Yangtze River Valley showed 10-50% yield loss due to pre-harvest sprouting when modern hybrid rice varieties were grown (Guo *et al.*, 2004; Tao *et al.*, 2007; Gao *et al.*, 2008). Low-lying estuary deltas are likely to be inundated more frequently as heavy rainfall events and tropical cyclones increase throughout the 21<sup>st</sup> century (IPCC, 2013), resulting in increased likelihood of damage to rice crops from flooding.

Rice cultivars with improved genetic resistance to pre-harvest sprouting can be bred and quantitative trait loci (QTL) associated with pre-harvest sprouting tolerance identified to support this (Lin *et al.*, 1998; Dong *et al.*, 2003; Guo *et al.*, 2004; Gao *et al.*, 2008; Hori *et al.*, 2010; Sasaki *et al.*, 2013), but this takes time. Another approach is to identify treatments to seed crops that reduce pre-harvest sprouting.

Molybdenum (Mo) has been reported to enhance seed dormancy. Tanner (1978) discovered Mo involvement in reducing pre-harvest sprouting in maize (*Zea mays* L.). In wheat (*Triticum aestivum* L.), Mo sprays at the flag leaf stage inhibited germination whilst dormancy increased with dose rate (0-600 mg Mo L<sup>-1</sup> as sodium molybdate) (Walker-Simmons, 1987; Modi and Cairns, 1994). Walker-Simmons (1987) also showed that endogenous abscisic acid (ABA) in embryos increased with molybdenum dose rate. The plant hormone ABA is associated with seed maturation and embryonic dormancy (Bewley and Black, 1994; Leung and Giraudat, 1998; Finkelstein *et al.*, 2002; Hilhorst, 2007; Bewley *et al.*, 2013), with the balance between ABA and gibberellic acid regulating seed dormancy and germination; GA is antagonistic to ABA and promotes germination.

The action of Mo in ABA production, and the subsequent enhancement of seed dormancy, has been shown in ABA-mutant plants across a wide variety of species (Walker-Simmons *et al.*, 1989; Leydecker *et al.*, 1995; Bittner *et al.*, 2001; Sagi *et al.*, 2002; Singh *et al.*, 2003; Porch *et al.*, 2006; Fang and Chu, 2008) including rice. Whilst the element Mo is biologically inactive in cells, it is a component of molybdenum-requiring enzymes (molybdoenzymes) and the molybdenum cofactor dependent enzymes oxo-molybdenum hydroxylase and aldehyde oxidase associated with carotenoid and phytohormone biosynthesis (i.e. indole-3-acetic acid and ABA) (Hille, 1996; Mendel and Schwarz, 1999; Mendel and Hänsch, 2002).

In maize and rice, precocious germination mutants fail to induce and maintain dormancy (Singh *et al.*, 2003; Porch *et al.*, 2006; Suzuki *et al.*, 2006; Fang and Chu, 2008). This is due to mutations in enzymes involved in ABA biosynthesis, including those involved in the synthesis of the molybdenum cofactor required for ABA biosynthesis. This cofactor combines with ABA aldehyde oxidase, the enzyme for the oxidation process that converts abscisic aldehyde into ABA (Mendel and Hänsch, 2002; Porch *et al.*, 2006).

Therefore, damage to rice seeds from pre-harvest sprouting following heavy rainfall or flooding might be reduced by foliar application of Mo before grain filling. The hypothesis that such application of Mo induces dormancy through its role in promoting ABA biosynthesis was tested here with glasshouse-grown plants submerged to simulate flooding for a period during seed development and maturation.

## Materials and methods

The Spanish Japonica rice cv. Gleva (from the Institute of Agrifood Research and Technology, Barcelona, Spain), susceptible to pre-harvest sprouting if flooded (Tejakhod and Ellis, 2018), was selected for this controlled-environment study at the Plant Environment Laboratory, University of Reading (51°27'N, 00°56'W) with pot-grown plants. The main investigation comprised a randomised block design with ten treatment combinations (four pots each) from five foliar treatments (i.e. Mo, ABA or deionised water) and submergence in water for four days at two stages of seed development, to simulate flooding, in three blocks. In each block, a further eight pots were included for parallel, contextual observations of seeds from plants neither sprayed nor submerged. Thus each block comprised 48 pots.

Seven seeds were sown in moist-growing media-filled plastic pots (180 mm-diameter, 3 L capacity with drainage holes) on 3 December 2013. The growing mixture comprised steam-sterilised coarse sand, crushed gravel, peat compost and vermiculite (2:4:1:4 v/v, respectively), with 3 kg m<sup>-3</sup> slow-release fertilizer (Osmocote Pro 3-4M, Everris International BV, The Netherlands) added. After sowing, the pots were maintained at a constant temperature of 25°C in the dark. Seedling emergence began nine days after sowing (DAS) and pots were transferred to three Saxcil growth cabinets (capacity 48 pots per cabinet) 10 DAS. The growth cabinets were maintained at 28/20°C day/night temperature with a photoperiod of 11 h d<sup>-1</sup>, 700 µmol m<sup>-2</sup> s<sup>-1</sup> light from cool white fluorescent tubes, a carbon dioxide concentration of 385 µmol mol<sup>-1</sup>, and day and night relative humidities of 60 ± 5% and 80 ± 5%, respectively. The pots were drip irrigated six times each day with the nutrient solution recommended by Yoshida *et al.* (1976). Seedlings were thinned to four plants per pot at the sixth leaf stage (36 DAS). Half of the main tillers reached anthesis by 11 March 2014 (98 DAS). This was recorded as zero days after anthesis (DAA).

Five foliar spray treatments comprising Mo at 100, 600 or 3000 mg L<sup>-1</sup> (as sodium molybdate (VI) dihydrate (Acros Organics, New Jersey, USA), ABA (grade ≥ 98%, Sigma Aldrich, UK) at 50 µM, or deionised (DI) water as the spray control were applied at 200 L ha<sup>-1</sup> at flag leaf appearance (84 DAS, 25 February 2014). Molybdate (MoO<sub>4</sub><sup>2-</sup>) was an appropriate form of Mo for this study since it can be used for plant growth and development (Lindsay, 1979; Mengel *et al.*, 2001; Kaiser *et al.*, 2005). Each treatment solution included 1% Tween 20 (Thermo Scientific, UK) as a surfactant to improve adherence to leaves. Plants were sprayed outside the growth cabinets and left to dry for two hours before returning to their previous position in the growth cabinet.

All pots were moved from the growth cabinets to a glasshouse maintained at 28°C with natural light at 16 DAA. The drip-fed irrigation was continued. Four-day submergence treatments were provided beginning 20 (31 March 2014) or 30 DAA (10 April 2014) within this glasshouse. Before each submergence treatment, the top of each pot was covered by steam-sterilised gravel to prevent the growing media being dislodged during submergence. Plants within each pot were staked to avoid lodging.

The submergence treatments, designed to simulate flooding, were provided in a wooden tank (2.4 × 1.2 × 1.1 m) lined with black polythene. This was filled with new tap

water before each treatment period in order to eliminate the possibility of contamination. Flooded water was circulated by a 180 W pump (RG25, Stuart-Turner, UK) and aerated with four 50-mm diameter, 14 W air-stones (Aqua One 12000, Kong's (UK) Limited) at each corner, throughout the four-day treatments to avoid anoxia. The water temperature was associated with air temperature, but not controlled directly. When submerged, all panicles were completely covered by water up to 0.8-0.95 m deep. The depth of water level was controlled to 20 mm above the highest panicle. After treatment, plants were returned to their previous position on the trolley in the glasshouse and irrigation resumed.

Irrigation ended 34 DAA (14 April 2014) and panicles harvested 37 DAA (17 April 2014) separately for each treatment within each block. Only filled seeds from each treatment were threshed from panicles. Sprouted seeds showing visible sign of the onset of germination (figure 1) were separated, counted and maintained as the sprouted seed sample within each treatment for each block.

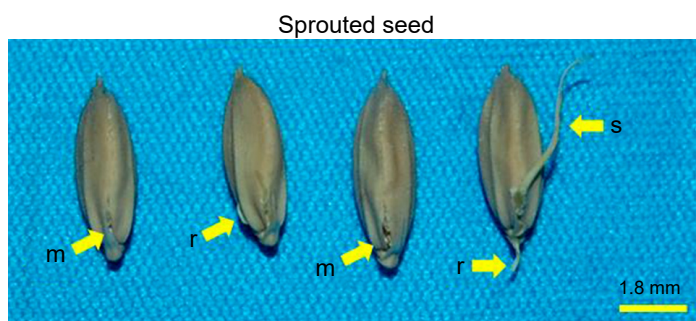


Figure 1. Mature, dry seeds of Japonica rice cv. Gleva showing different stages of visible signs of germination from pre-harvest sprouting from four days of submergence in water: enlargement of micropyle [m]; emergence of radicle [r]; emergence of shoot [s].

After harvest, seed samples were dried in a controlled-temperature room maintained at 20°C. Yield per plant, 1000 seed weight and the numbers of sprouted and non-sprouted seeds were determined. Yield per plant represented total weight of filled seeds produced from each treatment combination, in which the moisture content of sprouted and non-sprouted seed was close to 11% (water activity of all seed samples was  $0.51 \pm \text{s.d. } 0.05$ ).

For each treatment combination within each block, 5 g of seeds (at 11% moisture content) were ground separately in a grain mill. Between samples, the mill was brushed clean and fine dust removed by vacuum cleaner and aerosol spray to prevent cross-contamination between samples. The Mo concentration of these rice seed samples was determined following Official Method 999.10 (Association of Official Analytical Chemists, 2005). A weighed subsample of 0.5 g (*m*) of rice flour was placed in a 100 mL digestion vessel (SK-10 Medium/High Pressure Rotors, Milestone, Italy), previously washed with 10% v/v nitric acid (Merck, Darmstadt, Germany) and rinsed twice with purified water (18.2 MΩ, Milli-Q system, Millipore Co., Bedford, USA), 7 mL 0.1 M nitric acid (analytical grade 69-70%, Merck, Darmstadt, Germany) and 1 mL hydrogen

peroxide (30% m/v, Merck, Darmstadt, Germany) added, the vessel closed firmly with its cap and placed in a microwave oven (Ethos One, Milestone, Italy) programmed to heat the sample at 200°C for 20 minutes. The samples were then cooled to approximately 30°C. Blank samples were prepared as above, but without ground rice seed, and included in each microwave digestion batch.

The solution in the digestion vessel was then transferred to a 25 mL volumetric flask. The inner wall of the digestion vessel and lid was rinsed with deionised water. That solution was also added to the flask and the final volume adjusted to 25 mL with deionised water. Inductively Coupled Plasma-Mass Spectrometry (7700x ICP-MS, Agilent Technologies, US) was used to detect the amount of Mo in solution from (a) digested and (b) blank samples. Technical details of the instrument and operating conditions are reported in table 1. The final concentration of Mo ( $C$ ; mg kg<sup>-1</sup>) in each sample was calculated as:

$$C = \frac{(a - b) DF \times 25}{m}$$

where  $a$  and  $b$  are the concentration of Mo (mg L<sup>-1</sup>) in solution from digested samples and blank samples respectively,  $DF$  is the dilution factor which was 1 in this experiment (no dilution), 25 is the volume (mL) of sample solution analysed and  $m$  the original weight of the subsample (g).

Table 1. Inductively coupled plasma-mass spectrometry operating conditions for molybdenum determination.

Parameter	Value
LOD, LOQ	0.040, 0.075 mg kg <sup>-1</sup>
RF power	1500 W
Plasma gas flow-rate	0.91 L minute <sup>-1</sup>
Intermediate gas	0.32 L minute <sup>-1</sup>
Nebulizer	Babington Nebulizer
Nebulizer sample uptake	0.10 mL minute <sup>-1</sup>
Type of spray chamber	Quartx
Spray chamber temperature	2°C
Acquisition mode	- points peak jump
Total acquisition time	1 minute
Dwell time	0.1 second
Resolution	0.8 amu (atomic mass units)
Molybdenum standard solutions	0.5, 1, 5, 10, 25, 50 µg L <sup>-1</sup> prepared from 1000 mg L <sup>-1</sup> stock solution (Merck, Darmstadt, Germany)

Panicles with the same anthesis date from each treatment combination were sampled destructively just before and immediately after each submergence treatment on 20 or 30 DAA and 24 or 34 DAA, respectively. This was before harvest maturity and so included seeds that would have sprouted. In addition, panicles were also sampled from plants neither sprayed nor submerged at different times during seed development and maturation. On each date, one panicle per block was cut, snap-frozen for ten minutes using dry-ice and ethanol (99.5%, Sigma-Aldrich, UK), and individual panicles stored separately at -80°C for subsequent analysis of ABA.

To determine ABA, 40 filled-seeds were threshed from each panicle and ground to a fine powder using mortar and pestle with liquid nitrogen. Each ground sample was placed in a 15 ml centrifuge tube and then freeze-dried (GAMMA 1-16 LSC, Freeze Drying Solutions, UK) for four days. A 50 mg sub sample of freeze-dried rice seed was used for ABA extraction and detection by HPLC/MS-MS according to Forcat *et al.* (2008), but with two changes. First, the internal ABA standard (stable isotope-labelled compound,  $^2\text{H}_6$ , ABA-D6,  $0.5 \mu\text{g mL}^{-1}$ ) was reduced to 5 ng. Second, ABA extraction was carried out twice: the supernatant from the first extraction was collected, and the pellet re-extracted again to obtain the second supernatant. In order to obtain a pure extraction from mixed supernatant, it was filtered through a 1 mL sterile syringe (Plastipak, Becton, Dickinson and Company, USA) fitted with a filter (30 mm Syringe Filter  $0.45 \mu\text{m}$  Cellulose, Chromacol Ltd., UK). The latter had been wetted prior to use with LC/MS grade water (OptimaTM, Fisher Scientific UK Ltd.). The samples were stored at -20°C until analysis. Two blank samples without freeze-dried tissue were also prepared as above.

In addition to the above investigation, changes in seed ABA concentration during seed development and maturation in cv. Gleva were determined in a separate study. Seeds were sown on 9 May 2013 and the plants grown in pots (as above) in a temperature and photoperiod controlled heated and vented glasshouse ( $6.4 \times 8.6 \text{ m}$ ) with adjoining dark compartment ( $3.5 \times 2.1 \times 2.1 \text{ m}$ ). A short-day photoperiod (11 hours  $\text{day}^{-1}$ ) was provided in the latter from seedling emergence onwards: the plants on trolleys were moved from the dark compartments into the adjoining glasshouse at 0800 and back at 1900 each day. Temperatures were maintained at 28/20°C day/night (11 hours/13 hours) with synchronous thermoperiod and photoperiod until 10 DAA with ambient photoperiod thereafter. Half of the main tillers reached anthesis by 21 July 2013 (73 DAS; 0 DAA). Serial harvests of panicles were made at frequent intervals from 8 to 45 DAA, snap frozen, and then stored at -80°C. Seed ABA concentrations were determined as above at the same time as the main investigation.

Analysis of variance and linear regression analysis were performed using Genstat (13<sup>th</sup> Edition, VSN International, UK). The proportion of seeds showing pre-harvest sprouting was arcsine-transformed before ANOVA. The same three blocks were maintained throughout the growth cabinet – glasshouse – submergence – glasshouse sequence of the investigation. Observations from the additional plants neither sprayed nor submerged were excluded from analysis of variance and are reported separately (the deionised water spray treatment being the control in analyses).



## Results

Submergence for four days at 20 or 30 DAA reduced seed dry matter yield to only 1.5 or 1.8 g plant<sup>-1</sup>, respectively, compared with 4.5 g plant<sup>-1</sup> if neither sprayed nor submerged (figure 2A). Variation in seed yield amongst all submerged treatments was therefore small in relation to the reduction from submergence. Amongst the five foliar applications, there were no significant differences in seed yield for treatment at 20 ( $P = 0.148$ ) or 30 DAA ( $P = 0.402$ ), with the former providing almost uniformly lower yield, although this main effect was not significant ( $P > 0.05$ ). The main effect of foliar application approached significance ( $P = 0.065$ ), however, with seed yield poorest for deionised water.

Submergence promoted pre-harvest sprouting in the majority of seeds at 30 DAA and a minority at 20 DAA (figure 2B). Prior application of Mo or ABA reduced pre-harvest sprouting following submergence at 20 or 30 DAA. Significant main effects of time of submergence ( $P < 0.001$ ; 30 > 20 DAA) and foliar spray ( $P < 0.01$ ) were detected with no interaction ( $P > 0.25$ ). The main effect of foliar spray on pre-harvest sprouting was ranked deionised water > 3000 mg Mo L<sup>-1</sup> > 50 µM ABA > 600 mg Mo L<sup>-1</sup> > 100 mg Mo L<sup>-1</sup>; a Tukey test showed significant differences ( $P < 0.05$ ) between deionised water and each of 600 and 100 mg Mo L<sup>-1</sup>. The above ranking of foliar spray treatments effects was the same as that for the non-significant ( $0.10 > P > 0.05$ ) main effect on seed yield, namely deionised water < 3000 mg Mo L<sup>-1</sup> < 50 µM ABA < 600 mg Mo L<sup>-1</sup> < 100 mg Mo L<sup>-1</sup> (figure 2A).

The seed samples were divided into sprouted and non-sprouted seed fractions from treated plants. Amongst the foliar spray treatments, 1000-seed dry weight did not differ ( $P > 0.05$ ) between submergence at 20 or 30 DAA, or within either fraction amongst foliar spray treatments, but sprouted seeds were heavier (figure 3). All foliar spray and submergence treatments provided lighter seeds than plants neither sprayed nor submerged.

There was a positive relation ( $P < 0.001$ ) between Mo concentration in mature seeds at harvest and Mo applied at flag leaf appearance, notwithstanding considerable variation within each treatment level (figure 4). Analysis of variance confirmed this effect of foliar application ( $P < 0.001$ ) and a higher Mo concentration in sprouted than non-sprouted seeds ( $P < 0.001$ ). The Mo concentration in seeds neither sprayed nor submerged (0.76 mg Mo kg<sup>-1</sup>) or after ABA treatment and submergence (0.65-0.81 mg Mo kg<sup>-1</sup>) were similar and intermediate between the deionised water treatment mean and the regression line intercept at zero Mo applied (figure 4).

In the separate study sown on 9 May 2013, seed ABA concentration of cv. Gleva, neither sprayed nor submerged, declined exponentially during seed development and maturation (figure 5). Most of this decrease occurred before 20 DAA, with a stable plateau thereafter at about 90 ng ABA g<sup>-1</sup> seed.

In the 2014 simulated flooding study, seed ABA concentration also declined between 20 and 30 DAA, with mean values of 165.4 and 112.6 ng ABA g<sup>-1</sup> fresh weight obtained from seeds not sprayed and not destined for submergence. Analysis of variance of results following spraying and submergence showed a significant main effect of developmental duration ( $P < 0.001$ ) but not of foliar spray treatment or its interaction with developmental

duration ( $P > 0.05$ ). A Tukey test of the means for this significant main effect showed no difference between ABA concentrations at different times before submergence (20 and 30 DAA) or between different times after submergence (24 and 34 DAA) ( $P > 0.05$ ), but a significant decline with submergence (i.e. between these two cohorts,  $P < 0.05$ ) from 137.4 and 125.2 to 26.8 and 18.3 ng ABA g<sup>-1</sup> fresh weight, respectively. Every individual treatment comparison showed reduced seed ABA content after four days' submergence; the reduction varied from 64 to 92%.

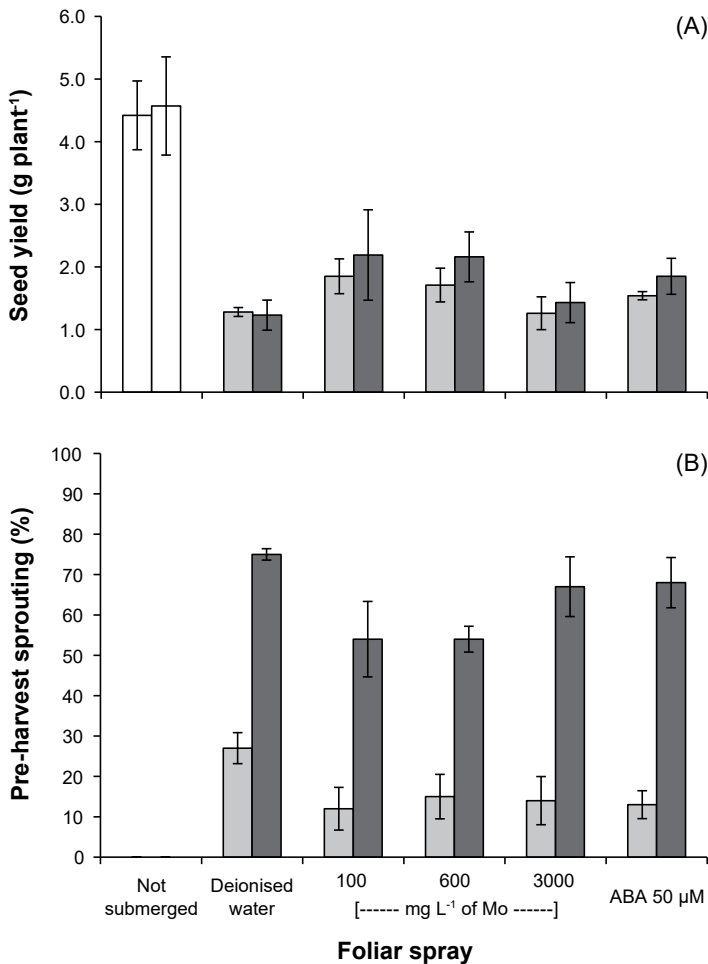


Figure 2. Effect of foliar application of deionised water, molybdenum (100, 600 or 3000 Mo mg L<sup>-1</sup>) or 50 µM abscisic acid (ABA), and subsequent submergence in water for four days from 20 (□) or 30 (■) DAA on (A) seed yield (g plant<sup>-1</sup>, at 11% moisture content) and (B) pre-harvest sprouting (%) of Japonica rice cv. Gleva. Seeds were harvested 37 DAA. The vertical bars represent mean  $\pm$  s.e. Results for plants neither sprayed nor submerged are also provided (□); no pre-harvest sprouting occurred in these plants.

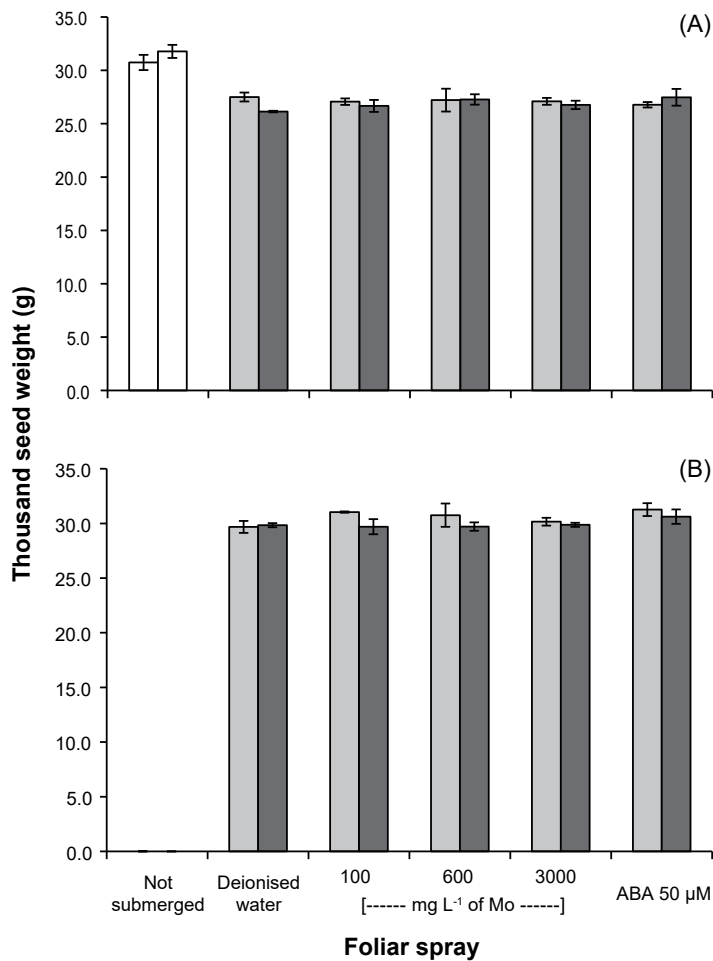


Figure 3. Effect of foliar application of deionised water, molybdenum (100, 600 or 3000 mg Mo L<sup>-1</sup>) or 50 µM abscisic acid (ABA) and submergence in water for four days from 20 (□) or 30 (■) DAA on thousand seed weight (g, at 11% moisture content) of (A) non-sprouted or (B) sprouted seeds of Japonica rice cv. Gleva. Seeds were harvested 37 DAA. The vertical bars represent mean ± s.e. Results for plants not submerged, in which no pre-harvest sprouting occurred, are also provided (□).

Those analyses included the effects of treatment with ABA, which provided similar seed ABA concentrations to the means above before (113.8 or 128.1 ng g<sup>-1</sup> fresh weight) and after submergence (30.6 or 14.3 ng g<sup>-1</sup> fresh weight), respectively. A subset of the dataset was analysed to test whether or not seed ABA concentration responded to Mo application rate (figure 6). There were no significant relations ( $P > 0.05$ ) between Mo application rate and subsequent seed ABA concentration before submergence at 20 (figure 6A) or 30 DAA (figure 6C) or after submergence at 34 DAA (figure 6D), whereas a negative relationship ( $P < 0.05$ ) was detected at 24 DAA after four days' submergence (figure 6B).

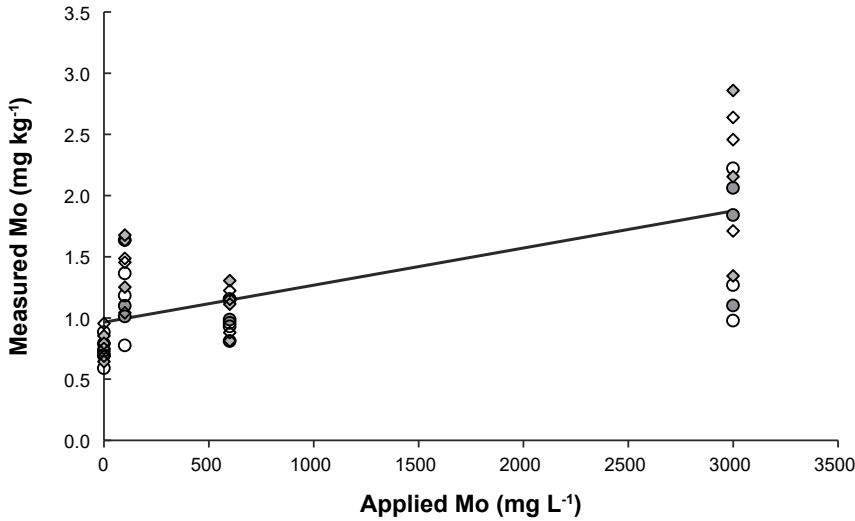


Figure 4. Linear regression ( $y = 0.952 + 0.000303 x$ ;  $R^2 = 0.461$ ;  $n = 48$ ,  $P < 0.001$ ) of the response of molybdenum (Mo) concentration ( $\text{mg kg}^{-1}$ ) of sprouted ( $\blacklozenge$ ,  $\blacklozenge$ ) or non-sprouted seed ( $\bullet$ ,  $\circ$ ) of Japonica rice cv. Gleva to applied molybdenum after submergence in water for four days from 20 (solid symbols) or 30 DAA (open symbols). Seeds were harvested 37 DAA and results for each of three blocks are shown. Seed from plants neither sprayed nor submerged had a mean seed concentration of  $0.76 \text{ mg Mo kg}^{-1}$ , whilst sprouted and non-sprouted seeds after submergence after treatment with  $50 \mu\text{M}$  ABA varied only from  $0.65$ – $0.81 \text{ mg Mo kg}^{-1}$ .

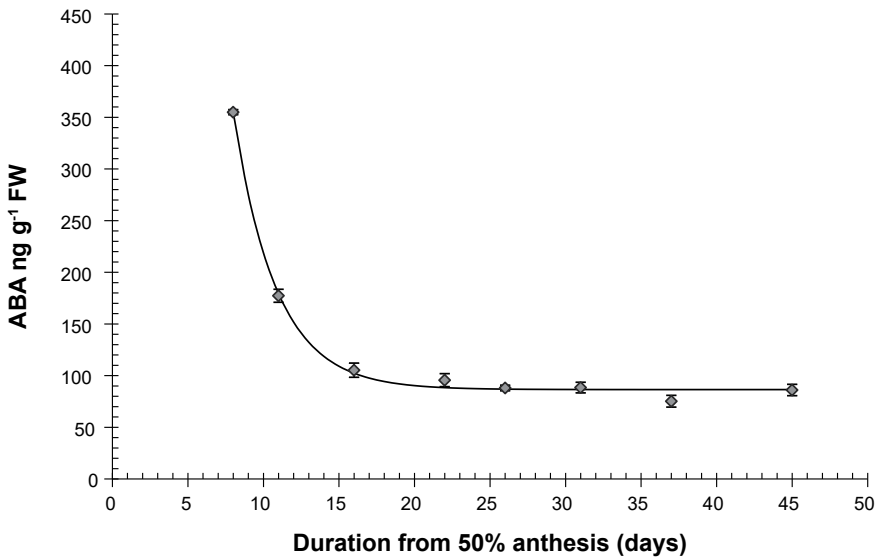


Figure 5. Decline in seed abscisic acid (ABA) concentration in Japonica rice cv. Gleva (grown in a controlled temperature and photoperiod glasshouse in 2013) during seed development and maturation; plants were neither sprayed nor submerged. The vertical bars represent mean  $\pm$  s.e. The fitted curve is quantified by the equation  $Y = A + B.R^X$ , where  $R = 0.7023$  (s.e. 0.0148),  $B = 4531$  (s.e. 779),  $A = 86.49$  (s.e. 2.45) ( $R^2 = 0.985$ ,  $n = 32$ ,  $P < 0.001$ ).

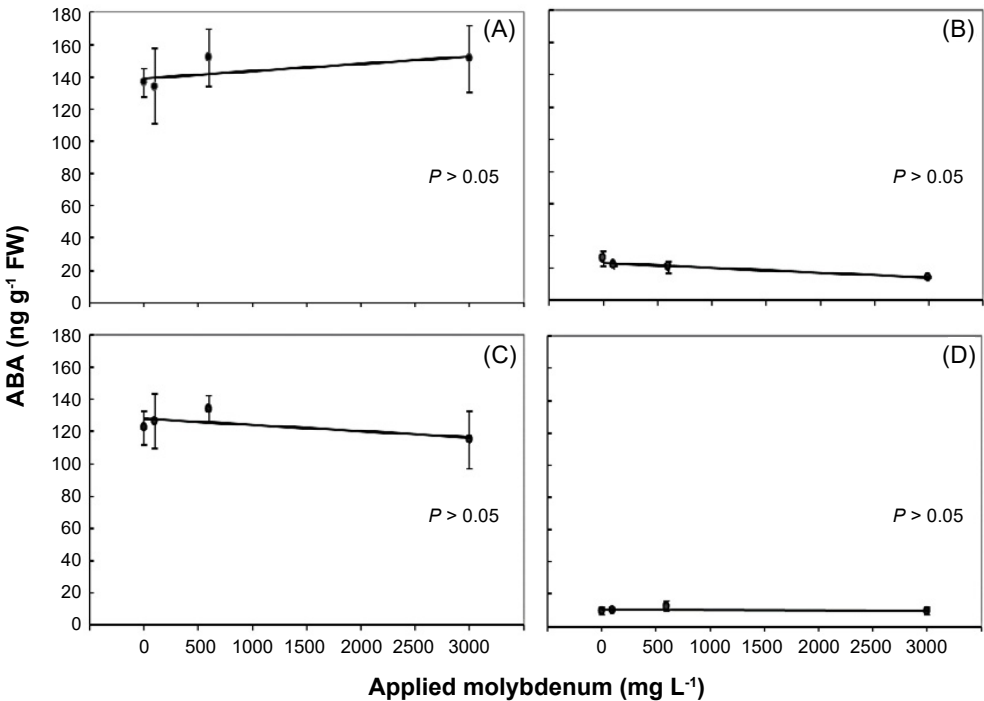


Figure 6. Effect of concentration of foliar applied molybdenum on subsequent seed abscisic acid (ABA) concentration of Japonica rice cv. Gleva rice (●) before (A, 20 DAA; C, 30 DAA) or after submergence in water for four days (B, 24 DAA; D, 34 DAA);  $n = 12$  in each case. The vertical bars represent means  $\pm$  s.e. Foliar application of abscisic acid at 50  $\mu\text{M}$  provided seed ABA concentrations of 113.8 (s.e. 8.57) and 128.1 (s.e. 6.98)  $\text{ng g}^{-1}$  at 20 or 30 DAA, respectively, before submergence and 30.6 (s.e. 8.53) and 14.3 (s.e. 1.97) at 24 or 34 DAA, respectively, after submergence.

Discussion

The 4-day submergence treatments applied here were successful in simulating flood damage to rice crops by reducing seed yield per plant (figure 2A), seed weight (figure 3), and stimulating considerable pre-harvest sprouting (figure 2B). Hence these results are in agreement with reports of severe losses in rice seed (from 10-100%) from flash flooding at various stages of crop development (Devender-Reddy and Mittra, 1985; Reddy *et al.*, 1985; Sharma and Ghosh, 1999; Kotera *et al.*, 2005; Kotera and Nawata, 2007; Das *et al.*, 2009; Singh *et al.*, 2009, 2011; Ray *et al.*, 2017). Similarly, the greater pre-harvest sprouting following submergence at 30 compared with 20 DAA (figure 2) is compatible with lower seed dormancy as crops approach maturity in general (Bewley and Black, 1994) and in cv. Gleva (Tejakhod and Ellis, 2018). The decline in seed ABA concentration during seed development and maturation (figure 5) is consistent with the expectation of ABA synthesis and accumulation during early seed development and its decline during seed maturation (Karssen *et al.*, 1983; Koornneef *et al.*, 1989; Fang and Chu, 2008).

The decline in ABA coincided with the decline in seed dormancy during seed development and maturation which resulted in greater pre-harvest sprouting after submergence at 30 than at 20 DAA (figure 2). Seed ABA concentration may be an incomplete explanation, however, because its major decline occurred early in seed development and maturation (figure 5) – and so largely before the first submergence treatment. This decline in seed ABA concentration overstates that for the embryo alone, due to assimilate deposition in the endosperm. Seed filling continues in cv. Gleva in this regime until 27-28 DAA (Tejakhod and Ellis, 2018). Comparison with those seed fresh weight increases confirms a net decline in ABA until 16 DAA. The role of ABA in seed dormancy and germination is equivocal on several grounds, with other factors such as seed covering structures and inhibitors also being relevant (Bewley and Black, 1999). This was the case in cv. Gleva: one third of seeds were able to germinate *ex planta* as early as 9 DAA in this regime if seed covering structures were removed, but none if not, in this comparatively low-dormancy cultivar (Tejakhod and Ellis, 2018).

Foliar application of Mo, as molybdate, to plants at the flag-leaf stage was also successful, with subsequent increase in Mo concentration of the rice seeds at harvest maturity with increasing dose (figure 4). This is in agreement with reports in other crops (Walker-Simmons, 1987; Jongruaysup *et al.*, 1994, 1997; Modi and Cairns, 1994). This uptake would be expected to improve ABA biosynthesis and so increase dormancy (see Introduction). The effect of Mo application on subsequent seed ABA concentration was not consistent, however (figure 6), and the main effect on seed yield per plant was only of borderline significance ( $0.05 < P < 0.10$ ). The reduction in pre-harvest sprouting was significant, however, and avoided much but not all of that caused by submergence. The most effective foliar spray concentrations in reducing damage to seed yield and in reducing pre-harvest sprouting were 100 and 600 mg Mo L<sup>-1</sup> (figure 2). The lower application rate would reduce potential risks from Mo toxicity, which results in poor plant development with yellow leaf tips, light-green shoots, and leaf malformation due to high accumulation of molybdo-catechol complexes in vacuoles (Liphadzi and Kirkham, 2006). No such symptoms were detected here, even at 3000 mg Mo L<sup>-1</sup>.

Foliar application of ABA provided some of the benefits derived from the Mo treatments for seed yield (submergence at 20 or 30 DAA) and pre-harvest sprouting (20 DAA only) (figure 2). Not surprisingly, there was no effect on seed Mo concentration at harvest maturity from ABA application (figure 4). Neither was subsequent seed ABA concentration affected significantly by ABA application. On the other hand, submergence reduced seed ABA concentration substantially overall (figure 6). This reduction in ABA and the big increase in pre-harvest sprouting (figure 2) was compatible with the role of ABA in the control of dormancy to prevent pre-harvest germination.

In conclusion, the considerable damage to seed yield and increase in pre-harvest sprouting caused by submergence, particularly from 30 DAA, was mitigated in part by application of Mo before anthesis, with application at 100 mg Mo L<sup>-1</sup> an effective dose to gain agronomic benefit. However, the considerable damage remaining despite this beneficial treatment points to the importance of plant breeding rather than or in addition to chemical spray treatments to combat damage to rice seed crops from heavy rainfall and flooding.

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